THE PERMEABILITY OF THE *ARBACIA* EGG TO AMMONIUM SALTS

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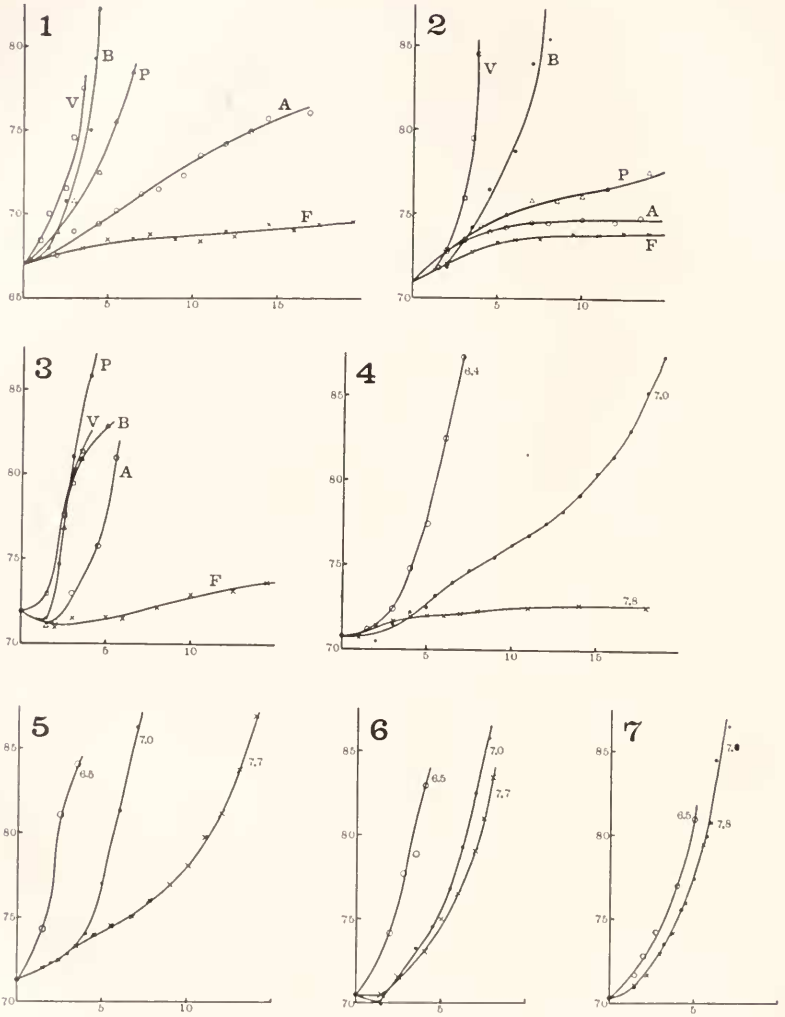
I

Salts, as a rule, appear to penetrate living cells with considerable difficulty. A striking exception to this behavior is found in the case of many ammonium salts which are known to enter mammalian erythrocytes with the greatest ease. (Gryns, 1896; Hedin, 1897). Jacobs (1927) has offered an explanation of this unusual property of the ammonium salts which is based on the behavior of the products of their hydrolysis in aqueous solutions. In the case of NH_4Cl, for example, the products of hydrolysis are largely undissociated ammonia, which enters all cells with the greatest ease, and highly dissociated hydrochloric acid, one of whose ions, Cl', is capable of being exchanged through the anion-permeable wall of the erythrocyte for OH'. The final result is a gradual accumulation within the cell of the salt in question, leading eventually to hemolysis. Other cells, which lack the pronounced specific permeability to anions that characterizes the erythrocyte, would not be expected to accumulate ammonium chloride, but only small quantities of free ammonia. In accordance with this view Jacobs finds that *Arbacia* eggs do not swell in isotonic solutions of ammonium chloride, the amount of ammonia which must penetrate in order to establish equilibrium with the external solution being too small to cause an appreciable volume change.

With the ammonium salts of the lower fatty acids, however, the situation is somewhat different. These salts form, upon hydrolysis, ammonia and free fatty acid. The latter is largely undissociated and is able to penetrate cells easily. Once inside them it unites with the ammonia, which has entered independently, forming salt again. Jacobs (1927) showed that the behavior of erythrocytes is in accordance with this theory, and he predicted that, since apparently all cells are permeable to both ammonia and the lower fatty acids, the ammonium salts of these acids should be able to penetrate any type of cell in this fashion. The present paper furnishes evidence that *Arbacia* eggs are freely



FIGS. 1–7: Swelling curves for *Arbacia* eggs in half molar solutions of various ammonium salts. Abscissæ, times in minutes; ordinates, diameters in arbitrary units each equal to 1.064μ. (See Footnote 1 concerning the pH values used.)

1. Ammonium formate (*F*), acetate (*A*), propionate (*P*), butyrate (*B*) and valerate (*V*); pH 7.0.
2. Same; pH 7.8.
3. Same; pH 6.5.
4. Ammonium acetate at pH indicated beside each curve.
5. Ammonium propionate at pH indicated beside each curve.
6. Ammonium butyrate at pH indicated beside each curve.
7. Ammonium valerate at pH indicated beside each curve.

permeable to these ammonium salts, and that the mechanism of the penetration is that predicted.

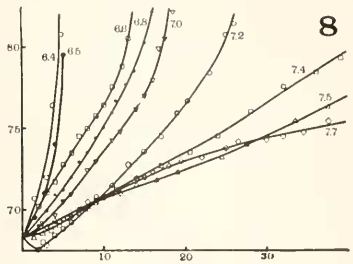


FIG. 8. The swelling of *Arbacia* eggs in M/2 solutions of ammonium acetate of the pH values indicated beside each curve. Abscissæ, times in minutes; ordinates, diameters in units each equal to 1.064μ. (See Footnote 1 concerning the pH values used.)

The solutions of ammonium salts were made up by adding to half normal NH_4OH sufficient concentrated acid to give the pH desired. The volume changes produced in this way were slight and the resulting solutions could be considered, with little error, to have a salt concentration of M/2, or approximately that of sea water. In some cases, where hypertonic solutions were desired, the salts were made up in bicarbonate-free sea water instead of distilled water. To obtain this, 2.4 cc. of normal HCl were added to a liter of sea water, and the CO_2 was driven off by aerating for twelve to fifteen hours. The pH was brought up to neutrality by the addition of a little NaOH and then the ammonium salt was prepared as before. The half molar solutions made up in distilled water were isosmotic with the cells, and penetration of salt from them could be detected by the swelling of the eggs, which proceeded until they were destroyed. The solutions in bicarbonate-free sea water, on the other hand, were initially hypertonic so that the eggs shrank, regaining their original size gradually only if the salt penetrated. The volume changes were studied by the method described in the preceding paper (Stewart, 1931).

II

The experiments were divided into two main series, one in which the ammonium salts were made up in pure M/2 solutions and another in which they were dissolved in sea water or half molar KCl. (NaCl could not be used because of its toxicity in unbalanced solutions.) The advantage of experiments of this second type lies largely in the fact that in them the eggs must approach, and then maintain, an equilibrium volume. Failure to do so gives definite evidence of injury to the cells.

This method was used as a check on the results obtained by the first one in which conditions were, on the whole, considerably more abnormal and for which no acceptable criterion of injury was available. The results obtained by the two methods were in good agreement.

For most of the experimental work the ammonium salts of the first five saturated fatty acids were used. Of the acids formed by the hydrolysis of these salts, four, namely acetic, propionic, butyric and valeric, are of very nearly the same strength. Furthermore, these four acids have dissociation constants that are almost the same as that of ammonia. Consequently at pH 7.0 there should be approximately equal amounts of free acid and ammonia present in the solutions. Formic acid is enough stronger so that the pH of the salt solution must be lowered to about 6.5 before equal quantities of acid and ammonia are obtained. For this reason the formate is not strictly comparable, at the same pH, with the other salts.

A comparison of the swelling curves of *Arbacia* eggs placed in M/2 solutions of the five salts at pH 7.0 (Fig. 1) shows the marked differences in the rates at which the salts enter the eggs. Since ammonia is known to penetrate these and other cells with extreme rapidity, it is reasonable to believe that the fatty acids, which in pure solutions enter cells somewhat more slowly, are to a certain extent the limiting factor which determines the rate of swelling. Because of the fact that at a given pH the concentration of free acid is approximately the same in the solutions of all of the salts except the formate, the swelling curves must give an indication of the relative order of penetration of the acids themselves. Numerous experiments indicate that the series is formic< acetic<propionic<butyric<valeric, which is the order of their lipoid solubilities, and the inverse of that for their molecular weights. Furthermore, it is the order frequently found in experiments with pure solutions of the acids, provided the results are not confused by injury effects. (For references to the literature see Jacobs, 1927).

The same order of penetration is observed if the salts are made up to half molar strength in either bicarbonate-free sea water or half molar KCl. Figure 9 gives a set of the curves obtained in experiments with sea water solutions. It will be noted that the valerate proves decidedly toxic, the eggs continuing to swell in it until they are cytolyzed. All of the other curves approach an equilibrium, however, indicating the lack of any decided injury to the cells. The fact that the equilibrium volume is not exactly the same as the initial volume of the eggs may be due to slight osmotic differences in the solutions themselves or may perhaps indicate the existence of secondary factors of some sort whose effects are of relatively minor importance.

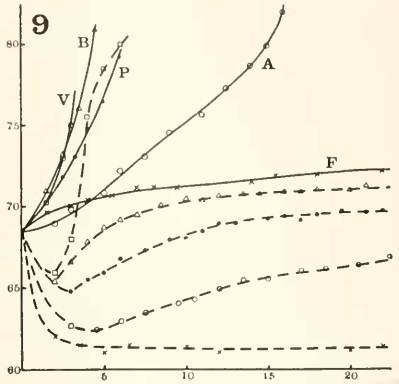


FIG. 9. Swelling curves for *Arbacia* eggs in solutions of ammonium formate (×), acetate (△), propionate (◎), butyrate (●) and valerate (□); pH 7.0. Solid lines, M/2 solutions; broken lines, M/2 solutions in bicarbonate-free sea water. (See Footnote 1 concerning pH values used.)

III

If the rate of entrance of free acid into the cell is the most important factor in influencing the rate of penetration of the salt, then it would be expected that the pH of the solution, which determines the relative concentrations of free acid and ammonia, would have a very important influence upon the swelling process. This proves to be the case. In general the increase in volume of the cells tends to become more rapid as the pH is lowered, down to about 6.0, and to become progressively slower as the pH is increased above 7.0. The swelling of the eggs in a solution of ammonium acetate at pH 7.8 is almost imperceptible (Fig. 2), while at pH 6.5 (Fig. 3)—an acidity which is not in itself markedly injurious—they increase in size so rapidly that cytolysis occurs within five or six minutes. A striking illustration of this effect of pH in changing the amount of free acid, and consequently the rate of swelling of the *Arbacia* eggs, is given in experiments such as that represented in Fig. 8. Here ammonium acetate solutions were used and the pH varied, in small steps, between 7.7 and 6.4. There is no exception to the orderly increase in rate with decrease in pH. The experiment has been repeated several times, always with the same results. Similar, though less extensive, observations have been made with each of the other salts (Figs. 5, 6, 7). The only essential difference between them lies in the spread of the three curves, which becomes less and less as the ease of penetration of the free acid involved increases, until finally, with the valerate, the curves are not separated from each

other by an amount that is significantly greater than the error of the method. As a result the three valerate curves do not maintain any definite relation to each other. Frequently penetration at pH 7.8 appears to be faster than at 7.0, or the curve for pH 6.5 is a trifle less steep than that for 7.0. But in about half of the experiments the order was found to be the same as that for the other salts, namely pH 6.5>pH 7.0>pH 7.8.

The effect of variations in pH is thus seen to parallel the changes in the amount of undissociated acid that is present in the solution. As has been mentioned previously, at pH 7.0 approximately equal quantities of free acid and ammonia are present. At a more alkaline reaction the amount of acid would be less than that of ammonia, and vice versa. Since the acid, rather than the ammonia, tends to be the limiting factor in determining the rate of swelling of the eggs, it is relatively easy to diminish this rate by a change of the pH in the alkaline direction. It is conceivable that at a sufficiently alkaline reaction almost no swelling would occur. This proves to be the case with an ammonium formate solution at pH 7.8, in which eggs often failed to swell appreciably during a 30-minute exposure. This failure was not due to any injury to the eggs, for after much longer exposures than that they were capable of giving typical swelling curves in an acetate solution. Attempts were made to obtain similar results with the other salts by raising the pH beyond 7.8, but toxic effects became so marked that the experiments could not be carried out.

As the pH is lowered below 7.0, more and more acid is present in the solution and swelling becomes increasingly rapid. If the pH is lowered sufficiently, the amount of ammonia might theoretically become so small that it would in turn assume the rôle of the limiting factor. This actually seems to be the case with the ammonium acetate. The maximum rate of swelling for this salt was obtained at about pH 6.0, while below that point the rate decreased until at pH 5.5 the curve obtained was almost identical with that at pH 6.5 or 6.6. Similar experiments with the other salts were impracticable because of the high toxicity of their more acid solutions.

As would be expected from the marked effect of pH upon the amount of free acid present in the solution, the divergence between the rates of swelling for the five salts is most marked in the alkaline range. Here the acids themselves are the chief factor in determining the rate, and differences in their ability to enter the eggs become most prominent. At pH 7.8 (Fig. 2) the penetration of the formate is sometimes almost impossible to detect, while the valerate enters so rapidly that cytolysis occurs in three minutes. At lower pH values, however, where the acids

are present in comparative abundance and where the ammonia becomes a more important factor, the difference would be expected to be less. Reference to Fig. 3 shows that as a matter of fact the spread of the curves at pH 6.5 is much reduced, the three for the propionate, butyrate and valerate being almost superimposed.

IV

In addition to the experiments which have been performed with the ammonium salts of fatty acids, a considerable number have been carried out with other ammonium salts and with some sodium and potassium salts of fatty acids. With ammonium nitrate and ammonium chloride no change occurred, within half an hour, in the volume of eggs placed in M/2 solutions, though eggs from the same lot were found to swell appreciably in ammonium formate at pH 7.7 during that time. Indeed, eggs may be left in ammonium chloride made up in bicarbonate-free sea water for 80 minutes without any change in size and at the end of this time give normal swelling curves if transferred to a neutral acetate solution. In other words, they appear not to have been injured appreciably by the chloride and their failure to swell is probably due to the fact that the acid cannot enter the cell and so no salt accumulates.

An attempt was made to work with solutions of ammonium benzoate and salicylate, but they proved very toxic to the eggs and it was not possible to obtain reproducible results with them. About all that can be said concerning these salts is that they both seem to enter the cells rapidly, though this apparent speed of penetration may be entirely due to the effects of injury which are ordinarily apparent within ten minutes. Ammonium lactate, on the other hand, enters very slowly, there being no appreciable penetration by the end of an hour from M/2 solutions at pH 7.7 or 7.0, though at pH 6.5 the volumes have increased to an extent that is just barely significant. At pH 7.0 a 10 per cent increase in volume occurs in about three hours as compared with a corresponding time of approximately five minutes for a similar solution of ammonium propionate. Since lactic acid differs from propionic acid only in the substitution of an OH group for a hydrogen atom, this behavior illustrates the marked effect upon permeability of the introduction of a polar group into a molecule.

Repeated experiments with sodium or potassium acetate and with sodium valerate at varying pH levels failed to show any penetration of these substances—or at least any penetration sufficient to cause a change in cell volume.

I wish to thank Dr. M. H. Jacobs, under whose direction this study has been made, for his many suggestions and his never-failing interest in the progress of the work.

Summary

1. The rate of swelling of *Arbacia* eggs in solutions isosmotic with sea water of the ammonium salts of the first five saturated fatty acids has been found to be in the order: valerate>butyrate>propionate>acetate>formate.

2. In solutions of these salts made up in bicarbonate-free sea water or in M/2 KCl recovery of the original volume after a preliminary shrinkage follows the same order.

3. Within the pH range from approximately 7.8 to 6.2 the rate of swelling increases with increasing acidity. Under certain conditions a further lowering of the pH may reverse the effect.

4. No significant changes in volume occur in solutions of ammonium nitrate or chloride, though cells exposed to such solutions for over an hour are still capable of swelling in ammonium acetate.

5. These results agree with the theory suggested by Jacobs, that there is no appreciable penetration of either the salt or its ions as such but that undissociated ammonia and fatty acid, formed by hydrolysis of the salt, penetrate the cell separately, uniting again after their entrance.

6. The rate of entrance of the acid, rather than that of the ammonia, usually appears to be the limiting factor in determining the rate of swelling of the cell.

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[1] Since the salt errors of the indicators for the solutions used are not exactly known, it has been thought best to use the uncorrected pH values throughout this paper, particularly since relative rather than absolute figures are of importance. The proper corrections may be made at any time when the necessary data become available. Since these corrections would probably be about −0.2, the values here given are too high by approximately 0.2 pH units. The standards used for comparison were the ordinary M/15 phosphate buffer solutions.